Cancer Target Discovery and Development (CTD^2)

Specific Aims

Institution: Dana Farber Cancer Institute

Specific Aims

Most human tumors, particularly those derived from epithelial cancers, exhibit global genomic alterations that make it difficult to identify mutations critical for cell transformation and to define the consequences of specific cancer-associated mutations. The application of whole genome approaches to large numbers of cancer specimens will eventually lead to a compendium of genetic alterations in specific cancers. Although this knowledge will provide a foundation to understand these cancers, these types of data do not provide insight into the function of the long list of genes identified by these approaches. The key challenge is now to develop and implement equally high throughput and efficient functional approaches that can provide biological insights into which of the myriad of genes implicated by these gene discovery efforts truly contribute to cancer initiation, tumor maintenance and/or metastasis. Until recently, the tools to perform such systematic functional studies were not available; however, the development of large RNA interference libraries, increasingly complete collections of human open reading frames (ORFs) and the tools to perform in vivo analyses of tumor populations now make it possible to pursue comprehensive approaches to identify and validate cancer targets.

In this Cancer Target Discovery and Development Center, we propose to establish a high-throughput functional pipeline to interrogate whether genes identified by TCGA as mutated in glioblastoma (GBM) and Ovarian (OVCA) cancers are essential and/or transforming. We will leverage established infrastructure at the Dana-Farber Cancer Institute to execute three inter-related aims using loss-of-function and gain-of-function screens in vitro and in vivo. We will make the outputs of these studies (data and methodologies) freely available to the scientific community and intend to participate in CTD^2 Network projects throughout the time frame of this project. We anticipate that this Center will provide the cancer research community with information that will facilitate the prioritization of targets based on both genomic and functional evidence, inform the most appropriate genetic context for downstream mechanistic and validation studies and facilitate the translation of this information into therapeutics and diagnostics.

Specific Aim 1. *Deploy genome-scale high-throughput "loss-of-function" screens to identify genes essential for GBM and ovarian cancer cell proliferation and viability*

In this Aim, we will employ a loss-of-function (LOF) approach using a genome-scale pooled RNAi library to identify genetic elements of interest (GEOIs) necessary for proliferation and/or viability in established human GBM or OVCA cells that have been comprehensively characterized at the genomic level. The cell lines will be selected based on their genomic profiles to represent major molecular subclasses defined by TCGA. At least 30 GBM and OVCA cell lines with adequate representation of these subclasses will be selected, optimized and screened for viability requiring genes by RNAi.

Specific Aim 2. *Deploy in vitro genetic screens to identify genomically altered genes with transforming activity in defined experimental models of GBM and ovarian cancer.*

In parallel, using a gain-of-function (GOF) approach, we will assay whether genes identified as mutated or amplified by TCGA contribute to increased proliferation and transformation. Here, we will use wild-type open reading frames (ORFs) for amplified candidates and engineered mutant ORFs in immortalized human astrocytes and ovarian serous epithelial cells. As in Aim 1, the target cells that will be used for these studies will represent the major GBM and OVCA molecular subclasses as defined by TCGA.

Specific Aim 3. Validation of candidates in *in* vivo experimental models

In this Aim, we will integrate the GOF and LOF data to define GEOIs that are essential for viability and/or transforming and then will perform *in vivo* genetic screens in defined contexts in appropriate microenvironment (e.g. orthotopic sites) to validate their biological relevance.

The outputs from this pipeline will be released publicly through our websites and the CTD^2 data portals, and the genome scale reagents will be made available through third-party distributors to enable further mechanistic and translational studies by the community.